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Growth and DNA Synthesis of Bacteriophage φX174 in a *dnaP* Mutant of *Escherichia coli*

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φX174*am3trD*, a temperature-resistant mutant of bacteriophage φX174*am3*, exhibited a reduced ability to grow in a *dnaP* mutant, *Escherichia coli* KM107, at the restrictive temperature (43°C). Under conditions at which the *dnaP* gene function was inactivated, the amount and the rate of φX174*am3trD* DNA synthesis were reduced. The efficiency of phage attachment to *E. coli* KM107 at 43°C was the same as to the parental strain, *E. coli* KD4301, but phage eclipse and phage DNA penetration were inhibited in *E. coli* KM107 at 43°C. It is suggested that the *dnaP* gene product, which is necessary for the initiation of host DNA replication, participates in the conversion of attached phages to eclipsed particles and in phage DNA penetration in vivo in normal infection.

Bacteriophage φX174 is a small, icosahedral phage containing a single-stranded circular DNA consisting of 5,386 nucleotides and 8 to 10 genes (1, 23). Phage multiplication starts with infection. The early events of infection with φX174 can be separated into attachment, eclipse, and complete penetration of phage DNA (15). Attachment of phage to the host cells is the reversible binding to the cell surface receptor and is followed by eclipse. The eclipse step is a process by which a phage loses its ability to infect cells as a result of a conformational change in the phage. DNA of the eclipsed particle is partially exposed (15); therefore, this particle is considered to be an intermediate in the penetration of phage DNA. Subsequent complete penetration of phage DNA into the cells is tightly coupled with the formation of parental replicative-form DNA (5). Most of the limited genetic information of the phage determines the coat proteins and their assembly; therefore, multiplication of φX174 depends almost completely upon host-mediated functions. On the other hand, to analyze the mechanism of DNA synthesis in *Escherichia coli*, many temperature-sensitive *dna* mutants which are defective in DNA synthesis at the restrictive temperature have been isolated. About 12 of these mutant genes have been found by genetic analyses. Most of the products of these *dna* genes have not been identified. Because multiplication of φX174 involves several discrete steps, it may be possible to identify the functions of *dna* genes by observing which steps in multiplication are blocked at the restrictive temperature.

A *dnaP* mutant of *E. coli* K-12 has been isolated among the mutants selected for β-phenethyl alcohol resistance at low temperature (30). The site of action of β-phenethyl alcohol may be the bacterial cell membrane (31). β-Phenethyl alcohol affects phospholipid metabolism (17, 18) and membrane-bound enzymes (11). The altered membrane presumably causes breakdown of the cellular permeability barrier (25) and conformational changes of membrane-associated proteins which are necessary for the initiation of DNA synthesis (13). It is suggested that the *dnaP* gene product is one of the membrane proteins involved in the initiation of DNA synthesis and that the *dnaP* mutation alters the membrane structure at the restrictive temperature (30).

In this work it is reported that multiplication of φX174 depends on this *dnaP* function and that the *dnaP* gene product is necessary for the eclipse and phage DNA penetration steps of φX174 infection.

MATERIALS AND METHODS

Bacteria and phage strains. All bacterial strains were derivatives of *E. coli* K-12. K12W6 is a wild-type strain. KD4301 [*uvrA6 thy phx*^{s} *trp*(Am) *tyr*(Am) *sup-126 his rif*^{r} *malA ilv*] is a φX174-sensitive mutant. KM107 is a *dnaP*(Ts) derivative of KD4301. The *dnaP*(Ts) gene was introduced into KD4301 from KY2901 (30) with P1*vir* by using *ilv* as the selected marker. KD48 [*uvrA6 phx*^{s} *trp*(Am) *tyr*(Am) *supD rif*^{r} *malA*] was used as a permissive host for φX174 amber mutants. φX174*am3* is a lysis-defective amber mutant in gene *E*, and φX174*am3trD* is a highly temperature-resistant mutant of φX174*am3*. φX174*am3trD* was isolated by nitrous acid treatment in our laboratory.

Media and buffers. TPG-CA medium was described by Sakai and Komano (20), and starvation

buffer (SB) was described by Denhardt and Sinsheimer (3). Borate buffer was 0.05 M sodium tetraborate solution, pH 9.5.

Chemicals. Lysozyme was purchased from Sigma Chemical Co. Mitomycin C was obtained from Kyowa Hakko Co., Ltd. [^{3}H]thymidine (23 Ci/mmol) was purchased from Daiichi Pure Chemical Co.

Culture methods. Bacteria were grown with shaking to 8×10^7 cells per ml at 30°C in 10 ml of TPG-CA medium containing 50 μg of thymine per ml (TPG-CA-thy medium). The cells were collected by centrifugation at 4°C for 15 min at 3,000 × *g*, washed twice with an equal volume of SB, and resuspended in 4 or 10 ml of SB.

Assay for intracellular phages. A sample (0.1 ml) from the infected culture was transferred into 0.9 ml of chilled borate buffer containing 0.5 mg of lysozyme per ml and 15 mM EDTA and allowed to stand for 30 min at 0°C. After vigorous stirring with a Vortex mixer for 1 min in the presence of several drops of chloroform, the phage in the sample was diluted with borate buffer and titrated by plating with indicator bacteria.

Kinetics of phage attachment. Host cells were grown, washed, and resuspended in 4 ml of SB as described above. Halves of the suspension were incubated for 15 min at 30°C and for 120 min at 43°C, respectively. φX174*am3trD* was added to each of them at a multiplicity of infection (MOI) of 0.4. At various intervals, attachment was monitored. The procedures were essentially the same as those described by Newbold and Sinsheimer (16).

Kinetics of phage eclipse. Host cells were grown, washed, and resuspended in 10 ml of SB as described above. Halves of the suspension were incubated for 15 min at 30°C and for 120 min at 43°C, respectively. φX174*am3trD* was added to each of them at an MOI of 0.2. At various intervals, eclipse was monitored. The procedures were essentially the same as those described by Newbold and Sinsheimer (16).

Penetration of phage DNA. The procedures were essentially the same as those described by Dumas and Miller (4). ^{3}H-labeled φX174*am3trD* was prepared by the procedure reported by Knippers et al. (10) except that [^{3}H]thymidine was used instead of ^{32}P in TPG-CA-thy medium. The specific activity of the purified phage was approximately 2×10^{-6} cpm/PFU. Host cells were grown with shaking to 10^8 cells per ml at 30°C in 40 ml of TPG-CA-thy medium. Mitomycin C (50 μg/ml) was added to the culture, and the mixture was incubated at 30°C for 15 min. Host cells were washed and resuspended in 2 ml of SB. Halves of the suspension were incubated for 120 min at 30 and at 43°C, respectively. The cell suspension incubated at 30°C was separated into two portions. ^{3}H-labeled φX174*am3trD* (0.1 ml) was added to one portion of this suspension (0.5 ml) at an MOI of 10; the mixture was incubated at 30°C for 5 min, and 0.4 ml of SB, prewarmed at 30°C, was added. The other portion of this suspension (0.5 ml) was treated as above; 0.4 ml of a twofold concentration of TPG-CA-thy medium, prewarmed at 30°C, was added to the mixture instead of SB. The cell suspension incubated at 43°C was separated into two portions and treated as above at 43°C. Each sample was incubated at 30 or 43°C for 30 min and chilled on ice. The cells were collected by centrifugation and washed five times with 1 ml of ice-cold borate buffer containing 6 mM EDTA. The final cell pellet was resuspended in 1 ml of the same buffer. The radioactivity in a 0.2-ml portion of the washes and a 0.2-ml portion of the resuspended cells was measured, and total radioactivity was computed.

Heat inactivation of phage particles. Heat inactivation was carried out as described by Haworth et al. (6).

Mitomycin C treatment. Host cells were grown, washed, and resuspended in 10 ml of SB. Mitomycin C treatment was carried out at 30°C in SB to inhibit specifically host DNA synthesis by the procedure of Lindqvist and Sinsheimer (12).

Extraction of phage DNA. The phage DNA was extracted with sodium dodecyl sulfate and cold phenol from purified phage particles or cell lysates (10).

Measurement of acid-insoluble radioactivity. Phage DNA synthesis was estimated by the incorporation of [^{3}H]thymidine into acid-insoluble material. The procedures were essentially the same as those reported by Sakai and Komano (20).

Neutral sucrose density gradient analysis of phage DNA. The sample of phage DNA and ^{32}P-labeled phage DNA marker was layered onto 4.5 ml of a linear gradient of 5 to 20% sucrose in 0.05 M Tris-hydrochloride (pH 7.4) containing 0.5 M NaCl and 3 mM EDTA. This gradient was centrifuged for 4.5 h at 36,000 rpm at 4°C in an RPS 40 T2 rotor of a Hitachi 65P preparative ultracentrifuge. After fractionation, radioactivity incorporated into acid-insoluble materials was measured as described above.

RESULTS

Nature of φX174*am3trD*. A φX174-sensitive *dnaP* mutant, KM107, was constructed to investigate the effect of *dnaP* gene function on the in vivo growth of φX174. It has been reported that phage φX174 cannot grow normally at 42 to 44°C in *E. coli*, although it grows normally at 40°C (2), and in KD4301, the parent strain of KM107, φX174 wild type and φX174*am3* could not grow at 43°C. Therefore, a temperature-resistant mutant of φX174*am3* which could grow normally at 43°C as well as at 27°C has been isolated and named φX174*am3trD*. It has been reported that a mutant of φX174 with altered capsid proteins exhibits altered inactivation kinetics and thermal stability (24); therefore, some properties of φX174*am3trD* were examined.

φX174 neutralization antibody reacts with the phage capsid protein (26); therefore, most capsid mutants may well exhibit altered antiserum inactivation kinetics. However, examination of the response of φX174*am3trD* to inactivating antisera showed no significant differences from φX174*am3* inactivation at both 30 and 43°C (data not shown). It seemed likely that φX174*am3trD* was serologically identical to φX174*am3*.

Heat inactivation of φX174*am3trD* was car-

ried out as described in the legend to Fig. 1. φX174*am3trD* was more stable than φX174*am3*. These data indicate that the φX174*am3trD* mutant is altered in a capsid protein. It has been reported that multiplication of capsid mutants of φX174 in *E. coli dna* mutants is different from that of φX174 wild type (4, 6, 28, 29); however, φX174*am3trD* was used in these experiments since φX174 wild type and φX174*am3* could not grow at 43°C even in KD4301.

Intracellular phage growth of φX174*am3trD* in KM107 and KD4301 at 43°C after incubation at 30 or 43°C. *E. coli* KM107 and KD4301 cells were infected with φX174*am3trD* and allowed to stand for 15 min at 30°C to complete phage attachment. The mixture was immediately shifted up to the restrictive temperature (43°C) by adding a twofold concentration of TPG-CA-thy medium and incubated at 43°C, and intracellular phages were assayed.

The results are shown in Table 1. The burst size (B/A) in KM107 at 30°C was about half that in KD4301 at 43°C after incubation at 30°C. These results were consistent with those of Ca^{2+}-dependent transfection experiments (data not shown). The growth of φX174*am3trD* was normal in a *dnaP* mutant strain at 30°C (data not shown).

To determine whether a *dnaP* mutant strain could support the growth of φX174*am3trD* under conditions where the *dnaP* gene function was previously inactivated, the host cells suspended in SB were incubated for 105 min at the restrictive temperature (43°C) and then infected with φX174*am3trD* at 43°C. The results are shown in Table 1. The burst size (B/A) in KM107 was about 100 times lower than that in KD4301. These data indicate that the host *dnaP* gene function is involved, albeit perhaps indirectly, in the growth of φX174*am3trD*.

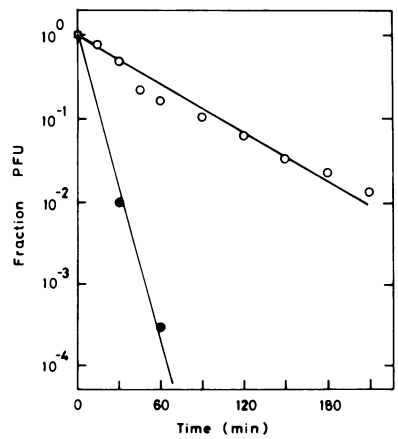

FIG. 1. *Heat inactivation of φX174am3 and φX174am3trD particles at 56°C. The experimental procedure is described in the text. Inactivation is expressed as the loss of PFU per milliliter versus incubation time at 56°C. Symbols: ●, φX174am3; ○, φX174am3trD.*

TABLE 1. *Intracellular phage growth of φX174am3trD in E. coli KM107 and KD4301 at 43°C after incubation at 30 or 43°C*[a]

Strain	Temp[b] (°C)	Phage (PFU/ml × 10^{-7}) At time of infection (A)	Phage (PFU/ml × 10^{-7}) At 120 min after infection (B)	Burst size (B/A)	Ratio of KD4301/KM107
KM107 [*dnaP*(Ts)]	30	1.0	220	220	2.4
KD4301 (*dna*$^+$)	30	1.0	520	520	
KM107	43	1.0	22	22	118
KD4301	43	1.0	2,600	2,600	

[a] The cells that were grown, washed, and resuspended in 4 ml of SB as described in the text were divided into two equal portions. One half (2 ml) of the cell suspension was incubated with aeration for 15 min at 30°C, and φX174*am3trD* prewarmed at 30°C was added to the cell suspension at an MOI of 0.1. After 15 min at 30°C without aeration, the mixture was shifted to 43°C by adding 2 ml of a twofold concentration of TPG-CA-thy medium (zero time), which had been prewarmed to 43°C, and was incubated with aeration at 43°C. The other half (2 ml) was incubated with aeration for 105 min at 43°C, and φX174*am3trD* prewarmed to 43°C was added to the cell suspension at an MOI of 0.1. After 15 min at 43°C without aeration, 2 ml of a twofold concentration of TPG-CA-thy medium (zero time), prewarmed to 43°C, was added to the mixture. The mixture was incubated with aeration at 43°C. At 120 min after the addition of the medium, a 0.1-ml amount of the sample solution was transferred into 0.9 ml of chilled lysis buffer, and the intracellular phage was titrated as described in the text.

[b] Incubation temperature before infection.

φX174*am3trD* DNA synthesis in KM107 and KD4301 at 43°C. The effect of the *dnaP* mutation on φX174*am3trD* DNA synthesis was examined in phage-infected cells by measuring the incorporation of [^{3}H]thymidine into phage DNA. Host cells were treated with mitomycin C, incubated in SB for 120 min at 43°C to inactivate the *dnaP* gene function before infection, and then infected with φX174*am3trD*.

The results are shown in Fig. 2 and 3. Host DNA synthesis was inhibited by mitomycin C treatment. φX174*am3trD* DNA synthesis in the parent strain (KD4301) occurred at a more rapid rate than in the *dnaP* mutant strain (KM107), and the amount of phage DNA synthesized in KD4301 was larger than that in KM107 (Fig. 2). Figure 3 shows neutral sucrose gradient sedimentation analyses of φX174*am3trD* DNA la-

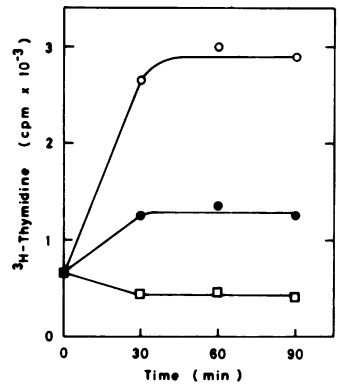

FIG. 2. *[^{3}H]thymidine incorporation into phage DNA in φX174am3trD-infected KM107 and KD4301 at 43°C after incubation at 43°C. Host cells were grown, treated with mitomycin C, and resuspended in 4 ml of SB as described in the text. The suspension was divided into two equal portions and incubated with aeration for 120 min at 43°C. φX174am3trD, prewarmed to 43°C, was added to one portion at an MOI of 2. After 15 min of incubation at 43°C without aeration, 2 ml of a twofold concentration of TPG-CA medium containing 20 μg of thymine per ml and 3 μCi of [^{3}H]thymidine per ml which had been prewarmed to 43°C was added to the suspension (zero time). The other portion was also treated as above without phage infection (zero time). Each suspension was incubated with aeration at 43°C. At the time indicated, a 0.1-ml amount of sample solution was transferred into 0.9 ml of 0.6 N NaOH at room temperature and incubated overnight at 37°C. After neutralization of the mixture with 0.1 ml of 6 N HCl, 1 ml of 10% ice-cold trichloroacetic acid was added in the presence of 200 μg of RNA per ml as a carrier. After the mixture was allowed to stand for 60 min at 0°C, precipitates formed were collected on a glass filter disk (Whatman GF/C). Radioactivity incorporated into acid-insoluble material was measured as described in the text. Symbols: ●, KM107; ○, KD4301; □, control, without phage infection.*

beled at 43°C all through the phage multiplication period. [^{3}H]thymidine was incorporated into progeny single-stranded DNA (fractions 15 to 17 in Fig. 3) and progeny replicative-form DNA (fractions 18 to 20 in Fig. 3). Consequently, single-stranded progeny DNA, as well as replicative-form DNA, was synthesized in both strains at 43°C. However, the amount of DNA synthesized in KM107 was much smaller than that in KD4301, although there was no qualitative difference between the DNAs synthesized. These results are consistent with the reduced phage yield (Table 1).

Attachment kinetics of φX174*am3trD* at 30 and 43°C. Since a mutation at the *dnaP* locus causes an altered bacterial membrane structure at the restrictive temperature (30), it is assumed that the partial inhibition of φX174*am3trD* DNA synthesis at 43°C in KM107 is due to a loss of an ability required early in infection and occurring on the cell surface. The early events in φX174 infection can be separated in vivo into attachment, eclipse (partial DNA injection), and complete penetration of phage DNA (15). When the cells starved for thymine or amino acids are infected with phage in SB, attachment and eclipse steps can proceed normally, but complete DNA penetration cannot (10, 15). Therefore, the effects of the *dnaP* mutation on φX174*am3trD* attachment were examined by measuring the reduction in the free phage titer at 30 and 43°C.

The results are shown in Fig. 4. The titer of the phage in the culture of the *dnaP* mutant strain KM107 decreased at the same rate as that in the culture of the parent strain KD4301 at

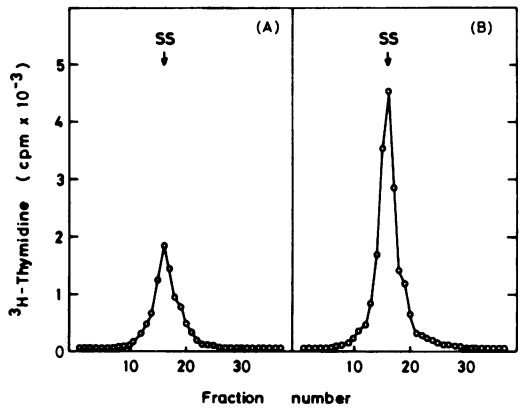

FIG. 3. *Neutral sucrose density gradient sedimentation of product DNA from φX174am3trD-infected KM107 and KD4301 labeled throughout phage DNA synthesis at 43°C. The conditions were the same as described in the legend to Fig. 2. At 60 min after the addition of medium, a sample (1 ml) was removed from the infected culture and suspended in 4 ml of chilled 0.05 M Tris-hydrochloride buffer (pH 7.4). The infected cells were collected and resuspended in 1 ml of the same chilled buffer. The labeled phage DNA was extracted as described in the text. Phage DNA extracted was precipitated by the addition of 2 volumes of 100% ice-cold ethanol. The mixture was kept at 0°C overnight. The precipitate was collected by centrifugation at 10,000 × g for 20 min at 2°C and dissolved in 1/10 standard saline citrate by stirring at 2°C overnight. The sample was sedimented through a linear sucrose gradient as described in the text. Fractions were collected from the bottom of the tube and diluted with 1 ml of 0.05 M Tris-hydrochloride buffer (pH 7.4) containing 0.5 M NaCl and 3 mM EDTA. A 1-ml amount of 10% ice-cold trichloroacetic acid was added in the presence of 200 μg of RNA per ml as a carrier. Radioactivity incorporated into acid-insoluble material was measured as described in the text. Arrows indicate the peak positions of added ^{32}P-labeled φX174am3 single-stranded (SS) DNA marker. (A) KM107; (B) KD4301.*

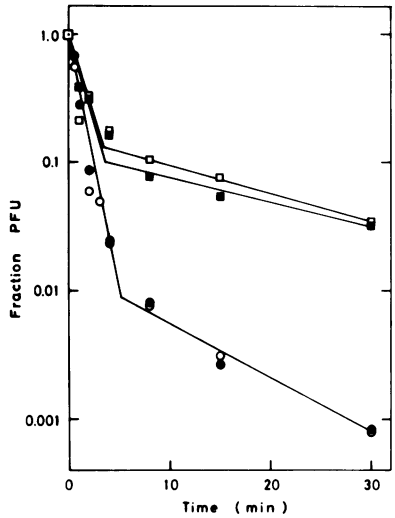

FIG. 4. *Attachment kinetics of φX174am3trD at 30 and 43°C. Attachment was monitored at 0, 1, 2, 4, 8, 15, and 30 min after the addition of phage. A 0.1-ml amount of sample was removed from the reaction mixture, diluted 100-fold with TPG-CA-thy medium at 0°C, and diluted further with the same medium at 0°C in a centrifuge tube containing 10^9 cells of E. coli K12W6 per ml as nonadsorbing carrier. Further attachment was stopped by the 10^3-fold dilution at 0°C. The cells were pelleted by centrifugation at 2°C, and the supernatant was titrated for residual phage as described above. Symbols: ●, KD4301, 43°C; ○, KM107, 43°C; ■, KD4301, 30°C; □, KM107, 30°C.*

both 30 and 43°C. KM107 and KD4301 exhibited the biphasic first-order attachment kinetics at both 30 and 43°C, and the rate constant (K) was 8×10^{-9} to 12×10^{-9} ml/min per bacterium. φX174*am3trD* phage particles were not inactivated for 30 min under the condition of attachment in the absence of cells (data not shown).

Since φX174*am3trD* phage particles could attach to the *dnaP* mutant strain as efficiently as to the parent strain at both permissive and restrictive temperatures, the host *dnaP* gene product does not appear to be required for φX174*am3trD* attachment.

Eclipse kinetics of φX174*am3trD* at 30 and 43°C. The effects of the *dnaP* mutation on φX174*am3trD* eclipse were examined by measuring the reduction in the free and infectious phage titers at 30 and 43°C. Eclipsed particles and uneclipsed particles can be eluted from attachment sites in the cell envelope with borate buffer containing 6 mM EDTA (borate-EDTA buffer). Since eclipsed particles are noninfectious, free and uneclipsed particles are titrated as infectious phage (15). The results are shown in Fig. 5. The titer of phage in the culture of KM107 decreased at the same rate as that in the culture of KD4301 at 30°C (Fig. 5A). However, there were significant differences between eclipse kinetics of φX174*am3trD* in KM107 and those in KD4301 at 43°C (Fig. 5B). The efficiency of phage eclipse in KM107 was 18% lower than that in KD4301 at 43°C (Fig. 5B). These data indicate that the *dnaP* mutant strain can eclipse φX174*am3trD* phage particles as efficiently as the parent strain at the permissive temperature; but φX174*am3trD* eclipse was inhibited in the *dnaP* mutant strain although it was normal in the parent strain at the restrictive temperature. Phage particles were not inactivated in 30 min under the condition of the eclipse reaction in the absence of cells (data not shown).

Penetration of φX174*am3trD* DNA in KM107 and KD4301. The effects of the *dnaP* mutation on φX174*am3trD* DNA penetration were examined by measuring ^{3}H-labeled phage DNA that could not be washed off the cell surface. As a control, we measured the amount of ^{3}H-labeled phage DNA that could not be washed off the surface of cells which had been

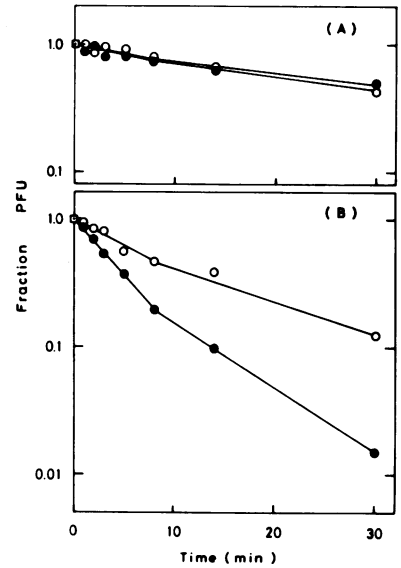

FIG. 5. *Eclipse kinetics of φX174am3trD at 30 and 43°C previously attached to KM107 and KD4301. Eclipse was monitored at 0, 1, 2, 4, 8, 15, and 30 min after the addition of phage. A 0.1-ml amount of sample was removed from the reaction mixture and diluted 100-fold with borate buffer containing 6 mM EDTA saturated with chloroform at 0°C to kill the infected cells. Further eclipse was prevented by the low temperature. The uneclipsed particles and eclipsed particles were eluted from the cells with borate-EDTA buffer. The titer of phage in borate-EDTA buffer represents residual and uneclipsed phage. (A) 30°C; (B) 43°C. Symbols: ●, KD4301; ○, KM107.*

starved for thymine and amino acids and infected in SB. Such cells allow phage attachment and eclipse, but not DNA penetration (15). The amount of phage DNA which had penetrated was calculated as follows: the control values were subtracted from the values measured after infection in TPG-CA-thy medium.

The results are shown in Table 2. The efficiency of the net penetration of φX174*am3trD* DNA in KM107 was 25% lower than that in KD4301. These data indicate that the net penetration of φX174*am3trD* DNA is partially inhibited in KM107 at 43°C.

DISCUSSION

φX174*am3trD* attaches normally to the *dnaP* mutant cells (*E. coli* KM107) at the restrictive temperature (43°C) (Fig. 4). However, phage eclipse and phage DNA penetration are partially inhibited in the *dnaP* mutant cells at 43°C (Fig. 5B and Table 2), and the phage can grow almost normally even in the *dnaP* mutant at 43°C after the early stages of infection proceed at the permissive temperature (30°C) (Tables 1 and 2). On the other hand, when the *dnaP* mutant cells were treated with glycine or chilled $CaCl_2$ to form permeable cells with or without preincubation at 43°C, phage DNA could penetrate into the host cells and the phage growth was normal at the restrictive temperature (27). The infectious DNA penetration in the transfection system may proceed by a mechanism different from that of phage DNA penetration in the normal infection system.

These results suggest that in phage infection the host *dnaP* gene product participates in the conversion of attached phages to eclipsed particles and in phage DNA penetration. Since φX174*am3trD* eclipse and phage DNA penetration are inhibited in the *dnaP* mutant at 43°C, phage DNA synthesis is reduced in the *dnaP* mutant and the phage is almost unable to grow under the same condition (Tables 1 and 2 and Fig. 2 and 3).

It has been reported that φX174*am3trD* and φX174*am3* can grow normally at the restrictive temperature in a *dnaI* mutant (21). The *dnaI* product is not necessary for φX174*am3trD* growth. In a *dnaB* mutant, φX174*am3trD* eclipse is normal, but the phage growth is inhibited at the restrictive temperature (data not shown). Although φX174*am3trD* is a capsid mutant of φX174*am3*, host dependency of φX174*am3trD* is similar to that of φX174*am3*.

The process of attachment is the reversible binding of the spikes of φX174 particles to the lipopolysaccharide receptor (9) in the cell envelope of *E. coli* in the presence of divalent cations such as Ca^{2+}. The process of eclipse requires a fairly high activation energy (36.6 kcal/mol) (16) and intact lipid A (9). During the eclipse stage, the attached phage binds more tightly and irreversibly and undergoes a conformational change (15). It has been reported that components other than the lipopolysaccharide receptor component of the cell membrane appear to be involved in

TABLE 2. *Penetration of φX174am3trD DNA in KM107 and KD4301*

Host cells and medium	Temp (°C)	cpm		Fraction penetrated [B/(A + B)]	Net penetration[a]		Efficiency of net DNA penetration (b/a)
		Total in washes (A)	Total in washed cells (B)		30°C (a)	43°C (b)	
KM107 [*dnaP*(Ts)]							
TPG-CA-thy	30	17,390	4,250	0.196	0.173		
SB	30	23,340	560	0.0234			0.207 (75)[b]
TPG-CA-thy	43	23,600	1,320	0.0529		0.0358	
SB	43	21,300	370	0.0171			
KD4301 (*dna*$^+$)							
TPG-CA-thy	30	16,900	3,710	0.180	0.157		
SB	30	22,600	530	0.0230			0.275 (100)
TPG-CA-thy	43	22,300	1,660	0.0693		0.0432	
SB	43	16,800	450	0.0261			

[a] Net penetration equals fraction penetrated in TPG-CA-thy minus that in SB.
[b] Numbers within parentheses indicate percentages.

phage eclipse (8, 14). The host *dnaP* gene product may be one of the components involved in phage eclipse and phage DNA penetration.

It has been reported that phage eclipse and further processes occur at a specific site in the cell envelope (9, 22). It seems that the host *dnaP* gene product, which is a membrane protein and necessary for the initiation of DNA replication in *E. coli*, catalyzes the processes of phage eclipse and phage DNA penetration. The involvement of the cell membrane in the initiation of replication of the *E. coli* chromosome has been suggested (7, 19); therefore, phage eclipse and phage DNA penetration may involve the initiation event of phage DNA synthesis in vivo in normal infection.

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LITERATURE CITED

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